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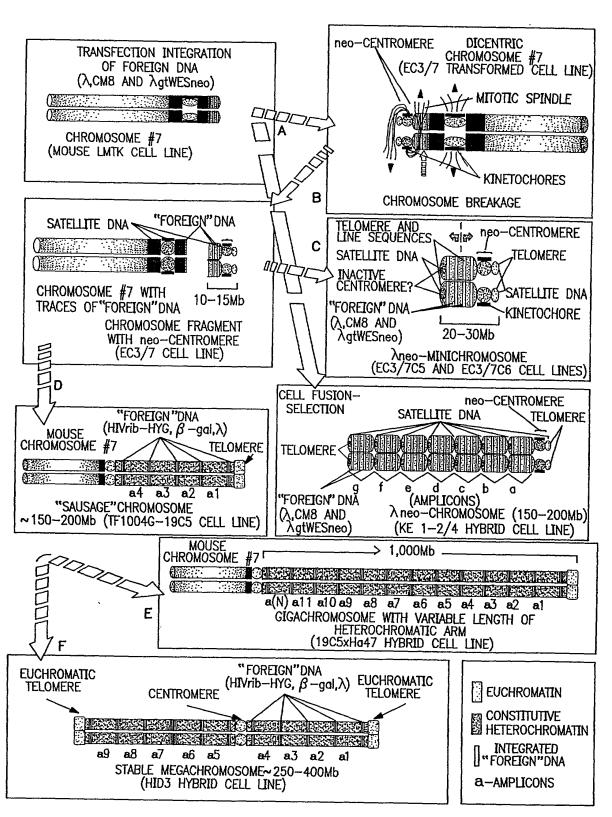


FIG. 2

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PRIMARY REPLICATION INITIATION SITE (MEGAREPLICATOR)

SECONDARY ORIGINS OF REPLICATION

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MEGAREPLICON OF THE CENTROMERIC REGION OF MOUSE CHROMOSOMES WITH TWO~7.5Mb TANDEM BLOCKS OF MOUSE MAJOR SATELLITE DNA (mSAT) FLANKED BY NON-SATELLITE DNA SEQUENCES

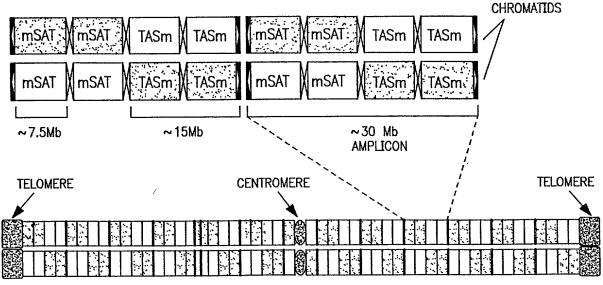
INTEGRATION OF "FOREIGN" DNA (pH132, pCH110, \lambda)



REPLICATION ERROR GENERATES INVERTED MEGAREPLICONS



AMPLIFICATION PRODUCES A TANDEM ARRAY OF IDENTICAL CHROMOSOME SEGMENTS (AMPLICONS) THAT CONTAIN TWO INVERTED MEGAREPLICONS BORDERED BY THE HETEROLOGOUS ("FOREIGN") DNA



STABLE MEGACHROMOSOME (~250-400Mb)

FIG. 3

EC3/7 MOUSE LMTK" (FIBROBLAST CELL LINE WITH neo-CENTROMERE) FUSION WITH CHO K20 CELLS AND SINGLE-CELL SUBCLONING SELECTION WITH G418 AND HAT KE1-2/4EC3/7C5 MOUSE LMTK FIBROBLAST MOUSE-HAMSTER HYBRID CELL LINE WITH THE neo-CELL LINE WITH THE MINICHROMOSOME AND THE STABLE \(\lambda\) neo-CHROMOSOME FORMERLY DICENTRIC **CHROMOSOME** COTRANSFECTION WITH PLASMIDS pH132 (ANTI-HIV RIBOZYME AND HYGROMYCIN-RESISTANCE GENES), pCH110 (lacZ GENE) AND \(\lambda\)cl 875 Sam7 (\(\rangle \text{PHAGE} \), SELECTION WITH HYGROMYCIN B TF1004G-19C5 MOUSE LMTK"FIBROBLAST CELL LINE WITH neo-MINICHROMSOME AND STABLE SAUSAGE CHROMOSOME FUSION WITH CHINESE HAMSTER OVARY CELLS (CHO K20 CELL LINE), SELECTION WITH HAT AND HYGROMYCIN B. 19C5xHa3 → RECLONING → 19C5xHa47 (CARRIES THE 19C5xHa4, GIGACHROMSOME) MOUSE-HAMSTER HYBRID CELL LINES CARRYING THE neo-MINICHROMOSOME AND THE SAUSAGE CHROMOSOME AND COUNTAINING A COMPLETE HAMSTER GENOME AND PARTIAL MOUSE GENOME BrdU TREATMENT, BrdU TREATMENT, SINGLE-CELL SINGLE-CELL CLONING, CLONING, SELECTION WITH SELECTION WITH G418, BrdU HYGROMYCIN B TREATMENT AND H₁D₃ RECLONING MOUSE-HAMSTER HYBRID G3D5 G3D6 CELL LINE CARRYING A MEGACHROMOSOME BUT NO MOUSE-HAMSTER HYBRID CELL LINES CARRYING: MINICHROMOSOME neo- MINICHROMOSOME ONLY FUSION WITH CD4+ MEGACHROMOSOME HeLa CELLS AND neo-RECLONE AND GROW MINICHROMOSOME CONTAINING neor, IN G418 RECLONE AND GROW SELECTION WITH G418 AND IN G418 AND HYGROMYCIN B ▼ HYGROMYCIN B H1xHe41 **GB43** GHB42 CARRIES MEGA-CARRIES neo-MOUSE-HAMSTER-

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CHROMOSOME WITH CD4 AND neor GENES; CONTAINS COMPLETE HAMSTER AND PARTIAL MOUSE GENOMES

CHROMOSOME AND

neo-MINICHROMOSOME

HUMAN HYBRID

CELL LINE CARRYING THE MEGACHROMOSOME AND A SINGLE HUMAN MINICHROMOSOME ONLY

FIG. 4

PUROMYCIN N-ACETYL TRANSFERASE -NhE I 1,075 - Xba I 1,345 V Kpn I 1,530 - Sst I 1,465 Sta 1,005 が Hind 田 345 SV40 EARLY PROMOTER -1,640 7 Nco I 225 MOND Not I 2,585 puro puc or (1.64Kb) (0,826.0) Sca I 3,185 (axto. E) Pst I 3,625 ~10,000 Sal I (1.1 kb)pTEMPUD ∼10kb URA (1.45kb) GGGATT 12 ~8,900 Eco RI 🔾 🔘 (axi.1) (1.05/80) (1.08/90.1) @ HUTEL ~5,100 Bgl II* SEQUENCE 0.3kb ~7,600 Eco RI O Bam HI, Bst XI ~6,100 Pst I 0 TARGET Pst I ~6820 Eco RI ~6550 Nco RI, Bam HI TKP-DT-A (790bp) Tk Promoter (270bp)

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FIG. 5